A Marsupial Oncovirus?

(Accepted 8 March 1979)

SUMMARY

A virus-like particle was observed in two continuous cell lines derived from the marsupial Sminthopsis crassicaudata (Fat-tailed Dunnart). The development of the particle was similar to the development of D-type oncoviruses. Initially, a crescent of nucleoid material was observed near the nucleus in the region of the Golgi apparatus. This crescent developed into a doughnut-shaped A-type particle which migrated through the cytoplasm towards the cell membrane where it budded either into a smooth membrane cytoplasmic vacuole or from the cell membrane. Only enveloped A-type particles were observed; no mature B-type, C-type or D-type particles were detected.

The virology of Australian marsupials has not received much attention so far, although a herpes virus has been isolated from the Parma wallaby, Macropus parma (Finnie et al. 1976), a pox virus has been observed in epidermal papillomata of the quokka, Setonix brachyrurus (Papadimitriou & Ashman, 1972) and a natural infection by the pox virus molluscum contagiosum has been observed in a kangaroo, Megaleia rufa (Bagnall & Wilson, 1974). At these laboratories numerous cell lines have been derived from Australian marsupials (Pye et al. 1977), some of which have been used in fusion and hybridization studies. During cell fusion studies (Graves & Hope, 1977), A-type particles were observed within the cells of the cell line CSL 235, which is derived from Sminthopsis crassicaudata (or Fat-tailed Dunnart, a mouse-sized marsupial carnivore that lives in southern Australia, feeding chiefly on grasshoppers). Further cultures of CSL 235 and of CSL 227, another cell line derived from the same species, have since been thoroughly examined for virus-like particles.

Confluent cultures of CSL 235 and CSL 227 were grown according to published methods (Pye et al. 1977). When prepared for this study, the CSL 235 cells had undergone more than one hundred population doublings while the CSL 227 cells had undergone less than ten doublings. The confluent cultures were dispersed with trypsin-versene and the cells re-suspended in phosphate-buffered saline. Pellets were formed by centrifuging the cell suspensions at 200g for 10 min. The resulting pellets were fixed (at room temperature for 24 h) in 2.5 %(v/v) glutaraldehyde, in 0.1 M-cacodylate buffer (pH 7.2) containing 2.5 mM-calcium chloride. After an overnight washing in the same buffer, the pellets were postfixed in 2%(w/v) osmium tetroxide in distilled water for 1 h at 0 °C and stained in 2%(w/v) uranyl acetate in distilled water for 2 h at room temperature. The pellets were dehydrated in an ascending acetone series and embedded in Durecupan ACM. Thin sections cut on an LKB Ultratome III and stained with lead citrate for 5 min at room temperature were examined with a Philips EM 301 electron microscope.

Virus-like particles were observed in both cell lines and appeared identical. In the cells observed, all stages of particle development appeared to be present. By comparison with the known developmental stages of typical C-type and B-type particles as described in standard texts (Dalton & Haguenau, 1973; Tooze, 1973; de Harven, 1974), it was possible to determine a sequence for the development of the various particles observed. The first observable stage in the development of this particle was a small electron dense crescent of core or nucleoid material that generally appeared in an area close to the nucleus of the
Fig. 1. Electron micrographs of thin sections of the A-type particles in CSL 235 cells. (a) A horse-shoe-shaped core is present; (b) several doughnut-shaped cores are present; (c) cores in various stages of development are present; (d) A-type particle budding from the cell surface; (e) A-type particle budding into a cytoplasmic vacuole; (f) a recently budded enveloped A-type particle with a tail.
cells in the region of the Golgi apparatus. The small crescent-shaped core developed progressively into a horse-shoe-shaped core (Fig. 1a) and finally a doughnut-shaped core (Fig. 1b). Presumably similar changes occurring in the third dimension result in the formation of a closed sphere. Such a doughnut-shaped core is usually considered a typical A-type particle (Bernhard & Guerin, 1958; Berhard, 1960; de Harven, 1974). In some clusters of particles all stages of development of the core could be seen (Fig. 1c). The doughnut-shaped A-type particle frequently seemed to be composed of two concentric shells (Fig. 1b). This has also been reported for A-type particles of murine leukaemia viruses (de The & O'Connor, 1966; de Harven, 1968).

The completed cores, or A-type particles, either accumulated at the site of assembly or migrated through the cytoplasm of the infected cell towards the cell membrane where they began to bud (Fig. 1d). Some budding appeared to occur into intracellular cytoplasmic vacuoles, (Fig. 1e). However, serial sections were not examined to exclude the possibility of these being invaginations of the cell membrane.

The A-type particle gradually protruded through the line of the cell membrane, until a hemispherical protrusion, represented in thin section as a semi-circular bleb, was seen (Fig. 1d). The A-type particle continued moving until it consisted of a circular knob connected to the cell by an isthmus of cytoplasm (Fig. 1e) which elongated and narrowed until the particle broke free from the cell. The newly budded particle was not perfectly round and often had a tail in the region whence it budded (Fig. 1f). The cell-free particle then rounded without condensation of the core in the isolated particle (Fig. 2). The most advanced stage of virus development that we have observed has been the enveloped A-type particle and particles with a morphology similar to mature B-type, C-type or D-type particles have not been observed.

While many cell lines have been derived from marsupials at these laboratories, most have a finite life span. Among the few exceptions are those lines derived from *Sminthopsis crassicaudata* (Pye et al. 1977) and it is in two of these that the A-type particles have been observed. However, not all of the marsupial cell lines have been examined with the electron
Although we have not specifically investigated whether the virus-like particles we have observed are of marsupial origin, this seems likely because they have been observed in cells at the stage of only five to ten population doublings. Limited attempts to grow the particles in cultures of human cells have been unsuccessful. Our classification of the marsupial virus-like particle as an oncovirus is based solely on morphological grounds.

We believe that the marsupial virus-like particles are members of the oncovirus subfamily of the retrovirus family because the morphology and development of the particles more closely resembles the oncoviruses than the spumiviruses and lentiviruses. Also the cells in which the particles developed did not undergo foamy degeneration or syncytium formation characteristic of the spumiviruses (Hooks & Gibbs, 1975) and there was no cytopathic effect characteristic of the lentiviruses (ter Meulen & Hall, 1978). We have been unable to classify the marsupial virus-like particles as a B-type, C-type or D-type virus, because no particle with a mature-type of morphology has been observed. The enveloped A-type particle may be the final product of virus development and in this respect the marsupial virus would resemble a virus observed in ESP-I cells, where the final product of growth is the enveloped A-type particle (de Harven & Sato, 1973). Because the budded A-type particles do not have a prominent fringe, typical of B-type particles, the development of the marsupial virus-like particles most closely resembles the development of D-type particles which also develop via budding A-type particles (Bykovsky et al. 1974; Yershov & Zhdanov, 1977). So far, all the D-type viruses which have been described have been observed in primate cells (Chopra, 1976; Heberling et al. 1977; Yershov & Zhdanov, 1977; Todaro et al. 1978). If the marsupial virus-like particle is in fact a D-type virus, it would be the first D-type virus observed growing within non-primate cells.

As the virus-like particle probably is of marsupial origin the known host range for oncoviruses has been extended to include the Metatheria (Marsupialia).

We thank Mr M. Kornitschuk for his ultramicrotomy.

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REFERENCES


*(Received 2 January 1979)*